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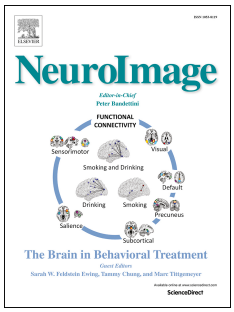

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Authorship

SM, FL, and LJ contributed to the design, set-up, maintenance and support of the Longitudinal Healthy Aging Brain (LHAB) database. BM performed the data analysis and wrote the first draft of the manuscript. All authors discussed the results, contributed to manuscript revision, read and approved the submitted version.

Functional Dedifferentiation of Associative Resting State Networks in Older Adults – a longitudinal study

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Abstract

Healthy aging is associated with weaker functional connectivity within resting state brain networks and stronger functional interaction between these networks. This phenomenon has been characterized as reduced functional segregation and has been investigated mainly in cross-sectional studies. Here, we used a longitudinal dataset which consisted of four occasions of resting state fMRI and psychometric cognitive ability data, collected from a sample of healthy older adults (baseline N = 232, age range: 64 - 87 y, age M = 70.8 y), to investigate the functional segregation of several well-defined resting state networks encompassing the whole brain. We characterized the ratio of within-network and between-network correlations via the well-established segregation index. Our findings showed a decrease over a 4-year interval in the functional segregation of the default mode, frontoparietal control and salience ventral attention networks. In contrast, we showed an increase in the segregation of the limbic network over the same interval. More importantly, the rate of change in functional segregation of the frontoparietal control ~~and the limbic~~ network was associated with the rate of change in processing speed. ~~and verbal learning and memory, respectively~~. These findings support the hypothesis of functional dedifferentiation in healthy aging as well as its role in cognitive function in elderly.

Keywords: resting state fMRI, brain networks, healthy aging, functional segregation, processing speed, ~~verbal memory,~~ longitudinal study

Introduction

Cognitive performance declines in normal aging, and this decline has been linked to a functional reorganization of the brain (Damoiseaux, 2017; Li et al., 2015). From the perspective of network neuroscience, the human brain can be regarded as a complex system driven by two main organizational properties: functional segregation and integration (Wig, 2017; Bullmore & Sporns, 2012). The first feature refers to highly clustered connectivity between regions forming subnetworks, most commonly identified in resting state fMRI studies as the default mode, executive control, attention, salience, sensorimotor, and visual networks (van den Heuvel & Hulshoff Pol, 2010; Smitha et al., 2017; Heine et al., 2012). On the other hand, functional integration entails connectivity between these subnetworks which enables global integration and distribution of neural information. Both characteristics are equally important, as shifting the balance between them can result either in fragmented information processing in isolated modules or in reduced functional specialization, and therefore lower resilience and increased vulnerability to disease (Fornito et al., 2015; Stam, 2014).

Current research suggests that older age is associated with the disruption and possibly the balance between these two properties (Damoiseaux, 2017; Chan et al., 2014; Betzel et al., 2014; Ferreira et al., 2016; Fjell et al., 2015; Geerligs et al., 2015). Most consistently, studies have shown a decrease in within-network connectivity and an increase in between-network connectivity – termed as functional dedifferentiation and implying more diffuse and less specialized patterns of functional connections (Damoiseaux, 2017; Antonenko & Flöel, 2013).

Current cross-sectional studies most frequently show functional reorganization primarily reflected in an implied decrease in within-network connectivity of the default mode network (DMN), and other associative networks such as the frontoparietal executive control network (FPCN) and the attention networks (Chan et al., 2014; Damoiseaux, 2017; La Corte et al., 2016; Onoda et al., 2012; Grady et al., 2016; Geerligs et al., 2015; Iordan et al., 2018; Meier et al., 2012). Simultaneously, these networks also show increased inter-network connectivity across age, simply summarized as higher functional connectivity strength usually between task-negative (i.e. DMN) and task-positive (i.e. FPCN, dorsal and ventral attention) networks (Ferreira et al., 2016; Spreng et al., 2016; Grady et al., 2016; Müller et al., 2016). Longitudinal studies seem to confirm these findings, as recent studies showed a non-linear decrease in within-DMN

connectivity (Staffaroni et al., 2018), and an increase in functional integration between DMN and FPCN, which was also related to lower processing speed (Ng et al., 2016).

The somatosensory networks (i.e. motor, visual, auditory) have been less sensitive to aging effects (Chan et al. 2014; Geerligs et al., 2015), although some cross-sectional studies do suggest higher within- and between-network connectivity of these systems in older compared to younger adults (Song et al., 2014.; Seidler et al., 2015; Meier et al., 2012). One study, specifically investigating the functional segregation, defined as the ratio of within- to between-network connectivity, showed that somatosensory networks do decrease in segregation, but that this change is more pronounced for the associative networks (Chan et al. 2014).

Current evidence points to the beneficial effect of functional segregation during resting state, as it has been related to better cognitive performance (Chan, 2014; Grady et al., 2016, Iordan et al., 2018, James et al., 2016), and greater cognitive improvement after training in older adults (Baniqued et al., 2018; Gallen et al., 2016). More specifically, lower segregation of associative networks has been related to worse episodic memory (Chan et al., 2014) and learning (Iordan et al., 2018), while lower integration between DMN and FPCN was associated to faster processing speed (Ng et al., 2016) and better cognitive performance during verbal learning (Geerligs et al., 2015). Nevertheless, longitudinal studies are essential to confirm the "true" aging-related changes in functional segregation and its relation to cognition independently of cross-sectional age. In longitudinal studies emphasis is on intra-individual change (in addition to inter-individual differences) as opposed to cross-sectional comparison, in which we can only investigate inter-individual differences, with a risk of confounding aging effects with cohort effects – individuals born in different eras my age differently (Schaie & Hofer, 2001).

Therefore, in this study we acquired four occasions of resting state fMRI and cognition measures. The main objective was to investigate the functional segregation of several well-defined resting state networks encompassing the whole brain. We hypothesized a decrease in segregation over a 4-year time span primarily comprising associative opposed to somatosensory networks. Finally, we assumed that higher segregation would be related to better performance in processing speed and verbal learning and memory.

Methods

Participants

Longitudinal resting state fMRI (rs-fMRI) data were taken from the Longitudinal Healthy Aging Brain Database Project (LHAB; Switzerland) – an ongoing project conducted at the University of Zürich (Zöllig et al., 2011). We used data from the first four measurement occasions (baseline, 1-year follow-up, 2-year follow-up, 4-year follow-up). The baseline dataset included 232 participants (age at baseline: M=70.8, range =64-87; females: 114). At each measurement occasion, participants completed an extensive battery of neuropsychological and psychometric cognitive tests and underwent brain imaging. ~~The brain imaging data was usually acquired in the same week as the behavioral assessments.~~ The brain imaging session was conducted in close temporal proximity to the behavioral assessments (difference between behavioral and MRI assessments in days (M±SD): baseline: 2.2±5.2, 1-y follow-up: 2.6±5.2, 2-y follow-up: 4.3±13.0, 4-y follow-up: 4.6±9.3).

Inclusion criteria for study participation at baseline were age ≥ 64, right-handedness, fluent German language proficiency, a score of ≥ 26 on the Mini Mental State Examination (MMSE; Folstein et al., 1975), no self-reported neurological disease of the central nervous system and no contraindications to MRI. Participation was voluntary and all participants gave written informed consent in accordance with the declaration of Helsinki. Self-reported physical and mental health of the sample at baseline, as measured by the SF-12 (Ware et al., 1996), were 50.9 ± 7.4 (M ± SD) and 54.8±6.3, respectively, which indicates above-average health compared to a ~~norm~~ normative population (Ware et al., 1998). As expected, sample means for these general health indicators slightly declined over time, but still indicated above-average health at 4y-follow-up (physical health score: 50.5±6.9, mental health score: 53.1±8.0, MMSE = 28.3±1.3).

At 4-y follow-up, the dataset still comprised 74.57% of the baseline sample (n = 173), of which 93% had complete data for rs-fMRI (see Table A.1). Participants remaining in the sample until the 4-year follow-up were compared to the full sample at baseline in order to estimate if there was selective attrition in the data (see Table A.2). The total selectivity was computed by standardizing the difference between the mean in the baseline sample and the 4-year follow up sample, on the standard deviation of the baseline sample in the variable of interest (Lindenberger, Singer, & Baltes, 2002). The size of the selectivity index was interpreted with reference to an effect size (Cohen, 1988). As can be seen in the Table A.2, total selectivity was

148 negligible for all measures (i.e., below the cut-off of 0.20 for a weak effect according to Cohen,
149 1988), suggesting that the participants remaining in the study at the 4-year follow-up did not
150 differ from the baseline sample in terms of age, education, physical and mental health.
151
152 **Cognitive measures**
153 Domain (average composite) scores were used to summarize neuropsychological performance
154 across several paper and pencil tests. Cognitive scores on individual tests were standardized to T-
155 scores (M = 50, SD = 10) with respect to baseline. If a cognitive domain was defined using
156 several cognitive tasks, T scores from separate tests were then averaged to calculate the domain-
157 specific average composite scores. For the calculation of cognitive scores, we excluded values
158 that were more than three median absolute deviations (MADs) above the median of the sample
159 distribution across measurement occasions (Leys, Ley, Klein, Bernard, & Licata, 2013).
160 Cognitive scores were calculated for three cognitive domains: processing speed, verbal learning
161 and verbal memory.

162 **Processing speed** was assessed using four psychometric paper-pencil tests: (1) the number of
163 correct responses across two test parts of the Identical Pictures Test (Kit of Factor-Referenced
164 Cognitive Tests; Ekstrom, French, Harman, & Dermen, 1976); (2) the number of correct
165 responses (within 2 minutes) on the Digit Symbol Test (Wechsler Intelligence Scale for Adults;
166 Von Aster, Neubauer, & Horn, 2006); (3) time in seconds, including the time used when an error
167 was made, needed to finish the Trail-Making-Test A (Reitan & Wolfson, 2004) (the scores were
168 reversed so the that higher scores equaled better performance), and (4) number of correct
169 responses (within 2 minutes) on the the LPS14, a subtest from the Leistungsprüfsystem 50+
170 (LPS), a German intelligence test developed to measure Thurstone's (1938) primary mental
171 abilities (Horn, 1983).
172
173 **Verbal learning and memory** were assessed with the *Verbal Learning and Memory Test*
174 (VLMT; Helmstaedter & Durwen, 1990), a test of serial list-learning with subsequent distraction,
175 recognition, immediate and delayed recall. The participants learned and recalled a list A
176 consisting of 15 semantically independent words over 5 trials. Then, the distractor list B
177 (consisting of 15 different words) was presented and recalled once. Finally, list A had to be
178 recalled without additional presentation of the words (immediate recall) and again after 20 – 30

minutes (delayed recall). The score from the delayed recall phase was used to define the **verbal memory** domain.

Verbal learning was defined using the total number of correct responses over 5 immediate free recall trials.

MRI acquisition

MRI scans were scanned on the Philips Ingenia 3T scanner equipped with a commercial 32-channel head coil array. T1-weighted (T1w) structural images were acquired using magnetization-prepared rapid gradient echo sequence (160 slices; TR= 8.1 ms, TE = 3.7 ms, FOV= 240 x 240 x 160 mm, flip angle = 8°, isotropic voxel size = 1.0 × 1.0 × 1.0 mm3). Two hundred and twenty-five multislice T2*- weighted volumes were retrieved with a gradient echo-planar sequence using transverse slice orientation (43 slices; voxel size: 3.5 x 3.5 x 3.5 mm3; TR = 2000 ms; TE = 21 ms; flip angle = 76°; FOV = 220 x 220 x 150 mm).

MRI preprocessing

Preprocessing was performed using the fmriprep BIDS app (v.1.05) (Esteban et al., 2019; Gorgolewski et al., 2017), a Nipype (Gorgolewski et al., 2011) based tool. Each T1w (T1-weighted) volume was corrected for INU (intensity non-uniformity) using `N4BiasFieldCorrection` v2.1.0 (Tustison et al., 2010) and skull-stripped using ANTs v2.1.0 (using the OASIS template). Spatial normalization to the ICBM 152 Nonlinear Asymmetrical template version 2009c (Fonov et al., 2009) was performed through nonlinear registration with the `antsRegistration` tool of ANTs v2.1.0 (Avants et al., 2008), using brain-extracted versions of both T1w volume and template. Brain tissue segmentation of cerebrospinal fluid (CSF), white-matter (WM) and gray matter (GM) was performed on the brain-extracted T1w using `FAST` (Zhang et al., 2001) (FSL v5.0.9). Functional data was slice time corrected using `3dTshift` from AFNI v16.2.07 (Cox, 1996) and motion corrected using `mcflirt` (FSL v5.0.9; Jenkinson et al., 2002). This was followed by co-registration to the corresponding T1w using boundary-based registration (Greve & Fischl, 2009) with 9 degrees of freedom, using `flirt` (FSL). Motion correcting transformations, BOLD-to-T1w transformation and T1w-to-template (MNI) warp

were concatenated and applied in a single step using `antsApplyTransforms` (ANTs v2.1.0) using Lanczos interpolation.

We used the CONN toolbox (v. 17f; Whitfield-Gabrieli & Nieto-Castanon, 2012) to regress out the nuisance covariates defined according to the 36-parameter model (Ciric et al., 2017): 6 motion parameters, signals estimated from cerebrospinal fluid and white matter, whole-brain global signal, their derivatives, quadratic terms, and squares of derivatives were regressed out from functional data separately for each run. Further, the rs-fMRI data was temporally bandpass filtered in the 0.01 – 0.1 Hz frequency range. We applied simultaneous filtering/nuisance regression, because it was shown to reduces correlation between time-series fluctuations and motion (Hallquist et al., 2013). In line with previous studies on healthy aging, global signal regression was performed, as this has been shown to be effective in minimizing the effects of physiological noise and head motion (Ng et al., 2016; Chan et al., 2014).

Network definition

The regions of interest were selected using a functional atlas composed of 200 cortical regions originally classified into 17 resting state network overlapping with the Yeo-Krienen atlas 17-network definition (Schaefer et al., 2018; Yeo et al., 2011). The choice of this atlas was made for consistency with past studies on functional connectivity changes with aging (Betzel et al., 2014; Ng et al., 2016). However, in order to increase the interpretability of our results, we regrouped the regions into 7 networks: default mode (DMN), dorsal attention (DAN), salience ventral attention (SVAN), frontoparietal control (FPCN), limbic (LIMB), somatomotor (SM), visual (VIS) networks.

Connectivity matrices for each of the participants and sessions were computed with pairwise correlation between average time series in selected regions of interest. These correlation coefficients were then transformed to z-values using the Fisher's r-to-z transformation. As it has been suggested that global signal regression can introduce “artefactual” anticorrelations (Murphy & Fox, 2017), negative z-values were excluded from the analysis, consistent with previous studies on healthy aging (Chan et al., 2014). Furthermore, the segregation index is more easily interpreted if calculated only with positive correlations, or separately for positive and negative correlations, as these two types of connections have different roles in between-network

connectivity. Therefore, this resulted in subject-specific 200x200 correlation matrices with diagonal and negative values set to zero.

The functional segregation index

The segregation of each of the resting state networks was quantified using a measure that summarizes values of within-network correlations in relation to between-network correlations (Chan et al., 2014). This metric is calculated according to the following formula: $(Z_w-Z_b)/Z_w$, where Z_w is the mean Fisher z-transformed r between regions within the same network and Z_b is the mean Fisher z-transformed r between regions of one network (e.g. nodes in the DMN) to all regions in other networks (e.g. nodes in the remaining networks: FPCN, DAN, SVAN, LIMB, VIS, SM).

Higher values of the segregation index reflect greater within- than between-network connectivity, and thus greater network segregation, while lower values represent a lower difference in within- to between-network connectivity and thus lower functional segregation.

Statistical analysis

Linear mixed effects analysis (*lme4* package (v. 1.1-18-1) in R (v. 3.5.2); Bates, Maechler & Bolker, 2012) was performed to assess the longitudinal change in the functional segregation of multiple resting state networks (i.e. DMN, DAN, SVAN, FPCN, LIMB, SM, VIS). As fixed effects, we entered time, age at baseline (grand-mean-centered variable), and their interaction term into the model. As random effects, we had intercepts for subjects, as well as by-subject random slopes for the effect of time. Gender (female = 1, male = 0) and education (on a scale from 1 to 3; 1 = high school with or without vocational education, 2 = higher education entrance qualification, business school or university of applied sciences, or 3 = university degree) were entered as nuisance covariates into the model, as current research suggest that these variables significantly relate to the topological organization of the human brain (Chan et al., 2018).

In the main models, we did not control for motion, as recent work suggests that this could remove true age-related connectivity effects (Geerligs et al., 2017, Staffaroni et al., 2018).

However, supplementary models were estimated by including motion as an additional nuisance covariate, defined as the average *framewise displacement* (FD) in a given measurement occasion.

Several other validation analyses, including additional covariates (e.g. mean functional connectivity strength, g~~ray matter volume~~ cortical thickness) have also been performed and are described in the Results section.

The same linear mixed effects models were performed to investigate longitudinal change in within network and pairwise between-network connectivity, and cognitive performance.

In addition, as it is very likely that the change in cognitive performance between the baseline and the 1-year follow-up assessment is influenced by the increased familiarity with the testing situation, reduction of anxiety, or general practice of relevant skills, we added a "retest effect" (baseline=0, 1-year follow-up=1, 2-year follow-up=1, 4-year follow-up=1) as a covariate in the linear mixed effects (LME) models for cognitive measures (Hoffman, Hofer, & Sliwinski, 2011; Oschwald et al., 2019).

Linear mixed models were fit by maximum likelihood and the p-values were obtained from the t-statistic using Satterthwaites's approximation to the denominator degrees of freedom (*lmerTest* package (v.3.0-1) in R (v.3.5.2); Kuznetsova et al., 2017).

The mixed models were fitted separately for each cognitive domain and resting state network. The results were adjusted for multiple comparisons using the Bonferroni correction.

Pearson's correlation analysis was performed to measure the strength of a linear association between the individual rate of change in cognition and the rate of change in the segregation of resting state networks. The correlation coefficients were calculated only for the cognitive domains and networks that showed significant longitudinal change according to the LME analysis.

The individual rate of change, defined as the subject-specific slope of the regression line between time and the cognitive scores/network segregation, was derived from the corresponding LME models described in this section (Ng et al., 2016, 2018). Therefore, the subject-specific trajectories of change in cognition and functional segregation of networks reflected the combination of fixed and random effects of time as defined in our models.

The significance of obtained correlation coefficients was corrected for multiple comparisons using the Bonferroni correction. The correlation analysis was performed using the R-based package *psycho* (v. 0.4.0.).

The results were visualized using the *ggplot* (v. 2-3.0.0) package in R.

Results

Change in the resting state network segregation

There was a significant decline in the segregation of associative networks such as the DMN (p=0.001), FPCN (p<0.001) and the SVAN (p=0.029) networks over the 4-y interval (Table 1). In contrast, there was a significant increase in the segregation of the LIMB network (p=0.005). The significant main effect of age at baseline indicated that older elderly have lower segregation of the DMN (p=0.001) and SVAN (p<0.001). For the latter we additionally found a significant interaction between time and age at baseline, reflecting a U-shaped association with respect to age and a turning point at around 75 years of age (Figure 1). This implies that after an initial decrease with age, from age 75 an upwards the segregation of this network increased.

Longitudinal aging effects in the segregation of the remaining three networks, the DAN (b < -0.0001, SE= 0.0016, 95% CI: -0.0031 – 0.0030; p= 0.976), VIS (b = 0.0005, SE= 0.0018, 95% CI: -0.0030 – 0.0041; p=0.768), and SM (b = 0.0003, SE= 0.0017, 95% CI: -0.0031 – 0.0037; p=0.878) networks, were not statistically significant (Table A.3.).

All statistically significant longitudinal aging effects survived multiple comparison corrections (corrected for 7 resting state networks, alpha=.05/7) except the main effect of time on the segregation of the SVAN.

Further, several validation analyses were done to test the robustness of presented results, as detailed below. More specifically, we investigated the effects of: 1) motion, 2) ~~gray matter volume~~ cortical thickness (CT), 3) mean functional connectivity, 4) global signal regression, 5) high-pass filtering, and 6) the choice of parcellation scheme (i.e. network definition), on the current analyses.

First, we included mean (within-session) framewise displacement (mean FD) as a covariate in the LME models. Although head motion was negatively related to segregation in the DMN and FPCN, and positively related to segregation in LIMB, the inclusion of motion parameters into the models did not qualitatively alter the results (Table A.4). Next, we conducted a supplementary analysis in which we excluded participants that had mean FD values that were more than three median absolute deviations (MADs) above the median of the sample distribution across measurement occasions. This did not qualitatively alter the results presented in the main manuscript (Table A.5).

Next, the inclusion of network-specific cortical ~~volume~~ thickness (calculated using FreeSurfer v. 6.0.0; (http://surfer.nmr.mgh.harvard.edu); Fischl, 2012), as an additional covariate in the LME models, did not significantly affect the main results (Table A.6), with the only exception being the time effect on the segregation of the SVAN, which was no longer significant after including cortical thickness (time*age effect was still significant). Hence, based on these additional analyses we are confident to conclude that changes in network functional segregation are not merely an effect of brain atrophy.

~~However, we did show an additional negative effect of age at baseline (does not survive multiple comparisons correction) on the segregation of the FPCN and DAN networks, suggesting that older participants have lower segregation in these networks when controlling for the network-specific GMV.~~

Further, the main findings remained largely consistent after controlling for mean functional connectivity, calculated as the mean connectivity strength across all connections in the unthresholded connectivity matrix (including absolute values of positive and negative correlation coefficients) (Table A.7). There was a significant negative effect of the mean FC on the segregation of the FPCN and LIMB networks, implying that participants with stronger global mean FC have lower segregation of these networks. However, the results remained comparable with the main findings, as there was a significant decline in the functional segregation of the DMN, FPCN and SVAN networks, and an increase in the segregation of the LIMB network, when controlling for mean FC.

Furthermore, similar findings were obtained after using different methodological approaches, such as repeating the analysis without global signal regression (Table A.8), and using a high-pass filter (0.008 Hz – Inf) instead of band-pass filtering as in main analyses (Table A.9).

However, in the analysis with high-pass filtered data, we did not find a significant time*age effect on the segregation of the SVAN, and there was a significant but weak age effect (i.e. does not survive multiple comparisons correction) on the segregation of the FPCN network.

Finally, to test if our results are contingent on the network definition found in the Schaefer et al. atlas, we reanalyzed our data using two other parcellation schemes available in the Power et al. (2011) and the Shirer et al. (2012; expanded version (Altmann et al., 2015)) functional atlases. Nonetheless, it should be noted that these parcellations have similar, but not overlapping, resting-state network assignments, as the two additional atlases include subcortical regions and do not

have a specific definition of the limbic network (please see the Appendix for more detailed explanation). Therefore, the validation analyses did not include the limbic network, as other atlases did not have a comparable definition of this resting-state network.

A significant decline in the functional segregation was once again shown in the default mode and salience networks (Table A.10-11). However, this decrease was not reproduced for the FPCN, indicating that the decline in functional segregation of this network is specific to the network definition found in the Schaefer et al. atlas.

Table 1. Longitudinal and cross-sectional aging effects in linear mixed effects models of the functional network segregation.

Network SI	*Predictors*	*Estimates*	*SE*	*CI*	*p*
	Gender	0.0423	0.0072	0.0282 – 0.0564	**<0.001**
	Education	-0.0006	0.0042	-0.0089 – 0.0076	0.885
DMN	**Time**	-0.0048	0.0014	-0.0076 – -0.0021	**0.001**
	Age	-0.0028	0.0008	-0.0045 – -0.0012	**0.001**
	Time * Age	0.0002	0.0003	-0.0004 – 0.0009	0.433
	Gender	0.0003	0.0066	-0.0126 – 0.0133	0.960
	Education	0.0019	0.0039	-0.0057 – 0.0095	0.629
FPCN	**Time**	-0.0053	0.0015	-0.0082 – -0.0025	**<0.001**
	Age	-0.0011	0.0008	-0.0027 – 0.0004	0.149
	Time * Age	-0.0001	0.0003	-0.0007 – 0.0005	0.746
	Gender	0.0204	0.0073	0.0061 – 0.0347	**0.006**
	Education	-0.0003	0.0043	-0.0087 – 0.0080	0.936
SVAN	**Time**	-0.0037	0.0017	-0.0070 – -0.0004	**0.029**[a]

	Age	-0.0041	0.0009	-0.0058 – -0.0024	<**0.001**
	Time * Age	0.0010	0.0004	0.0003 – 0.0017	**0.008**
	Gender	-0.0131	0.0095	-0.0317 – 0.0054	0.167
	Education	-0.0059	0.0055	-0.0167 – 0.0050	0.290
LIMB	**Time**	0.0057	0.0020	0.0017 – 0.0096	**0.005**
	Age	0.0011	0.0011	-0.0010 – 0.0033	0.303
	Time * Age	-0.0006	0.0004	-0.0015 – 0.0002	0.151

Note. Only the significant models are shown. Statistically significant effects ($p < 0.05$) appear in bold. [a] - does not survive multiple comparison corrections (alpha=.05/7). Abbreviations: SI = segregation index, DMN= default mode network, FPCN = frontoparietal control network, SVAN = salience ventral attention network, LIMB = limbic network.

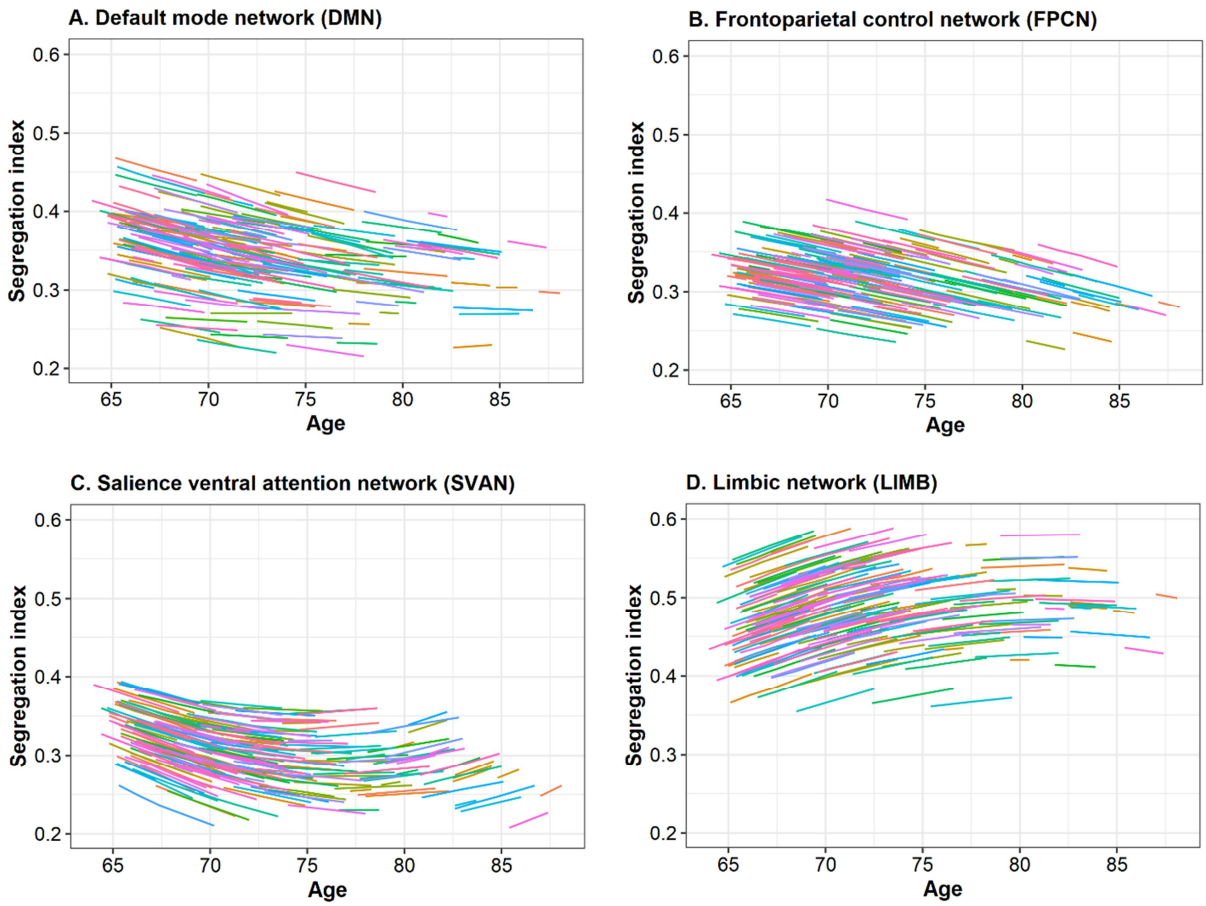

Figure 1. Spaghetti plots of model-fitted subject-specific trajectories of functional segregation values. There was a significant decline in the segregation of DMN (A) and FPCN (B). Changes in the segregation of SVAN seem to be U-shaped in regard to baseline age (C). There was an increase in the limbic network segregation across time (D).

Change in within- and between- network connectivity

To gain a better insight into what is driving the observed changes in functional segregation of various networks, we separately calculated the within- and between-network connectivity strength of all resting state networks.

The full presentation of the results outlined below can be found in tables A.12-14.

Within-network connectivity

We found a significant decrease in the functional connectivity strength of FPCN (b = -0.0022, SE = 0.0008, 95% CI: -0.0038 – -0.0006; p= 0.006). Also, there was an increase in the functional

connectivity strength of LIMB (b = 0.0061, SE = 0.0017, 95% CI: 0.0028 – 0.0094; p=<0.001) and SM (b = 0.0043, SE = 0.0017, 95% CI: 0.0009 – 0.0077; p=0.013).

Finally, we found a significant effect of age at baseline on intra-network connectivity, with older participants having lower connectivity strength within DMN (b = -0.0012, SE=0.0005, 95% CI: -0.0022 – -0.0002; p=0.014) and SVAN (b = -0.0018, SE = 0.0006; 95% CI: -0.0029 – -0.0006; p=0.003).

Of all the reported results, the time effect on the within-network connectivity strength of the FPCN and LIMB, and the effect of age at baseline on the connectivity strength of SVAN, survived the Bonferroni correction (alpha =.05/7) (see table A.12).

Between-network connectivity

Inter-network connectivity was increased between DMN and SVAN (b = 0.0019, SE = 0.0006, 95% CI: 0.0007 – 0.0031; p=0.002), and LIMB and SVAN (b = 0.0018, SE = 0.0007; 95% CI: 0.0004 – 0.0031; p=0.010).

Further, all of the networks that showed significant change in functional segregation also had an increase in connectivity strength with the SM network (see table A.14). More specifically, FPCN (b = 0.0020, SE= 0.0006, 95% CI: 0.0008 – 0.0032; p=0.001), DMN (b = 0.0026, SE = 0.0006, 95% CI: 0.0014 – 0.0038; p=<0.001**)**, SVAN (b = 0.0025, SE = 0.0009, 95% CI: 0.0008 – 0.0042; p=0.004), and LIMB (b = 0.0022, SE = 0.0008, 95% CI: 0.0006 – 0.0038; p=0.007), all had increased connectivity strength with SM over the 4-year interval.

In addition, DAN (b = 0.0029, SE = 0.0012, 95% CI: 0.0006 – 0.0053; p= 0.013), also showed an increase in between-network connectivity with SM.

Finally, the VIS network had an increase in functional connectivity strength only with the LIMB network (b = 0.0020, SE= 0.0010, 95% CI: 0.0001 – 0.0039; p=0.036).

Additionally, older participants had higher DMN-DAN (b = 0.0006, SE=0.0003, 95% CI: 0.0001 – 0.0011; p=0.015), LIMB-SVAN (b = 0.0010, SE = 0.0003, 95% CI: 0.0004 – 0.0016; p=0.001), and FPCN-SM between-network connectivity (b = 0.0009, SE = 0.0003, 95% CI: 0.0003 – 0.0014; p=0.003).

Of all the reported results, the time effect on the connectivity strength between DMN-SVAN, FPCN-SM, DMN-SM, and the effect of age at baseline on the connectivity strengths between

LIMB-SVAN, survived the Bonferroni correction (alpha =.05/21) (see table A.13 and table A.14).

Change-change association between brain and cognition measures

We found a statistically significant decline in performance in the three cognitive domains: processing speed (p<0.001), verbal learning (p<0.001) and verbal memory (p<0.001) (Figure A.2). There was a significant retest effect for processing speed (p<0.001) (Table 2). All statistically significant aging effects survived multiple comparison corrections (corrected for three cognitive domains, alpha=.05/3).

Table 2. Longitudinal and cross-sectional aging effects in the linear mixed effects models of cognitive performance.

Cognitive domain	*Predictors*	*Estimates*	*SE*	*CI*	*p*
	Retest	1.4413	0.3441	0.7669 – 2.1157	<**0.001**
	Gender	-0.5143	0.9628	-2.4013 – 1.3727	0.594
Processing speed	**Education**	1.2733	0.5617	0.1724 – 2.3741	**0.024**[a]
	Time	-0.4530	0.1180	-0.6842 – -0.2217	<**0.001**
	Age	-0.6676	0.0971	-0.8579 – -0.4773	<**0.001**
	Time * Age	-0.0485	0.0194	-0.0864 – -0.0105	**0.013**
	Retest	0.9806	0.6453	-0.2842 – 2.2454	0.129
	Gender	5.4023	1.1550	3.1386 – 7.6661	**<0.001**
Verbal learning	**Education**	1.4298	0.6741	0.1085 – 2.7511	**0.035**[a]
	Time	-1.0142	0.2090	-1.4239 – -0.6045	**<0.001**
	Age	-0.6085	0.1149	-0.8338 – -0.3832	**<0.001**
	Time * Age	-0.0966	0.0334	-0.1621 – -0.0311	**0.004**

	Retest	-0.6566	0.6864	-2.0019 – 0.6886	0.339
	Gender	3.9128	1.2060	1.5490 – 6.2766	**0.001**
Verbal memory	**Education**	1.7456	0.7024	0.3690 – 3.1222	**0.014**
	Time	-0.8192	0.2242	-1.2585 – -0.3798	<**0.001**
	Age	-0.4516	0.1224	-0.6916 – -0.2117	<**0.001**
	Time * Age	-0.1178	0.0358	-0.1880 – -0.0477	**0.001**

Note. Statistically significant effects ($p < 0.05$) appear in bold. [a] - does not survive multiple comparison corrections (alpha=.05/3).

Based on the longitudinal change results, twelve Pearson's correlation analyses were performed, assessing the change-change relationship between the processing speed, verbal learning, verbal memory and the functional segregation of the DMN, FPCN, SVAN, and LIMB networks.

There was a positive correlation between the rate of change in the segregation of the frontoparietal network and processing speed ($r(226) = 0.15$, 95% CI [0.021, 0.27], $p = 0.023$), indicating more significant decline in processing speed at higher rates of decline in the segregation of the FPCN network (Figure 2.). In addition, we performed a regression analysis in which we included age as a covariate, to test if the association between the rate of change in cognition and brain was influenced by age at baseline. The effect of change in the segregation of the FPCN on the rate of change in processing speed remained significant ($\beta = 0.15$, 95% CI [0.02 – 0.28], $p = 0.024$), while the effect of age was not significant ($\beta = 0.00$, 95% CI [-0.01 – 0.01], $p = 0.94$). These effects remained across all processing pipelines, except in the one which involved high-pass instead of band-pass filtered data (figure A.4.A). The reported association did not survive multiple comparison correction (corrected for 12 models, alpha = .05/12).

Further, we found a significant positive association between the rate of change in the segregation of the limbic network and verbal learning ($r(226.00) = 0.18$, 95% CI [0.05, 0.30], $p = 0.008$), and verbal memory ($r(225) = 0.23$, 95% CI [0.098, 0.35], $p = 0.001$), indicating lower rates of decline in verbal learning and memory at higher rates of increase in the segregation of this network. However, these results were inconsistent across different preprocessing pipelines, with

either null associations or even negative effects of segregation of the limbic network on verbal learning and memory (figure A.4.B and A.4.C). Given that the initial positive association between the limbic network and cognitive performance was not replicated across the control analyses, we interpreted it as unstable and removed it from the final discussion. For further details on these findings please see the Supplementary information. The rate of change in other networks was not significantly associated to the rate of change in cognitive performance (Appendix – Brain-cognition change-change association).

~~The association between the rate of change in processing speed and the rate of change in the segregation of DMN (r (226) = 0.080, 95% CI [-0.051, 0.21], p > .1), SVAN (r(226) = -0.080, 95% CI [-0.21, 0.050], p > .1), and LIMB (r(226) = 0.0084, 95% CI [-0.12, 0.14], p > .1) networks, was not statistically significant. The rate of change in verbal learning was not significantly related to the rate of change in the segregation of FPCN (r(226) = -0.10, 95% CI [-0.22, 0.03], p > .1), DMN (r(226) = -0.05, 95% CI [-0.18, 0.08], p > .1), or SVAN (r(226) = -0.02, 95% CI [-0.15, 0.11], p > .1) networks. Finally, the rate of change in verbal memory was not significantly related to the rate of change in the segregation of FPCN (r(225) = -0.042, 95% CI [-0.17, 0.089], p > .1), DMN (r(225) = -0.059, 95% CI [-0.19, 0.072], p > .1), or SVAN (r(225) = -0.034, 95% CI [-0.16, 0.097], p > .1) networks.~~

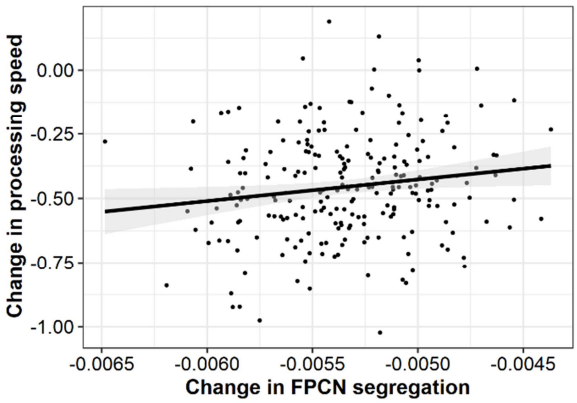

Figure 2. Change-change association between the segregation of the FPCN and cognitive performance. Decline in in the segregation of the frontoparietal control network was associated to decline processing speed (r (226) = 0.15, 95% CI [0.021, 0.27], p = 0.023).

Discussion

In the present study we quantified the functional segregation of seven resting state networks using a metric defined as the ratio of within- to between-network connectivity. As hypothesized, our findings showed a disruption in the segregation of associative resting state functional networks across a time span of 4 years. This was reflected as a decrease in the segregation of the default mode, frontoparietal control and salience ventral attention networks (i.e. with a U-shaped cross-sectional trajectory), and an increase in the segregation of the limbic network. Further analysis, in which we looked at pairwise inter-network interactions, indicated a complex pattern of decrease and increase in within- and between-network connectivity. Finally, decline in the segregation of the frontoparietal control network was related to decline in processing speed~~, while increase in limbic network segregation was related to less decline in verbal memory~~ over the 4-year interval.

Our results are in line with previous cross-sectional studies implying a decline across age in the segregation of associative, and to lesser degree somatomotor and visual networks, which, here, did not show a significant decline in the overall functional segregation (Chan et al., 2014; Grady et al., 2016). Furthermore, our findings are compatible with a recently published longitudinal study, with comparable age range, showing a specific decline in the segregation of higher-order networks, namely the default mode, salience/ventral attention, and control networks (Chong et al., 2019).

The segregation index has been shown to be more sensitive than other graph theoretical metrics in relation to age-related changes in functional organization (Chan et al., 2014). Moreover, this measure is not limited to pairwise analysis of between-network connectivity, but it can detect global modifications in functional connectivity patterns and thus provide us with a summary measure per desired network.

In the present study, we see that the observed pattern is not specific to individual networks, but is rather generalizable across associative networks, pointing to a dichotomy between two large systems – associative and somatosensory, suggesting that the former is more vulnerable to aging effects (Chan et al., 2014; Geerligs et al., 2015).

Further examination of within- and between-network connectivity revealed associative networks (i.e. DMN, FPCN, DAN, SVAN and LIMB were not significant after multiple comparisons correction) had increased connectivity with the somatomotor network over the 4-year interval.

Interestingly, the somatomotor network had sustained functional segregation across this period, which is probably due to the preserved connectivity strength within this network, maintaining the balance to its between-network connections. Further, this increase in between-network integration is consistent with earlier research indicating a diffuse increase in positive correlations in older compared to young adults, especially between somatosensory and other networks, such as the DMN, executive control and attention networks (Ferreira et al., 2016; Geerligs et al., 2015; Song et al., 2014; Iordan et al., 2018; Betzel et al., 2014; Meier et al., 2012). Similar findings, that is the increase in somatomotor between-network connectedness, were shown in the cross-sectional study of Song and colleagues (2014), interpreted as the reorganization of hub nodes (i.e. highly connected brain regions), as hubs in the DMN became non-hubs in older participants, and hubs in the somatosensory gained hubness and greater role in inter-network connectivity. Overall, a greater interdependence of these two types of systems is consistent with the hypothesis that the associative networks compensate for the decline in sensorimotor function that occurs in older age (Li and Lindenberger, 2002; Seidler et al., 2010, 2015).

Further, we showed significant change in the segregation of three higher-order networks, DMN, SVAN and the FPCN, in line with studies showing functional reorganization primarily in these networks in older adults compared to young (Damoiseaux, 2017; Grady et al., 2016; Chan et al., 2014; Meier et al., 2012, Betzel et al., 2014). More specifically, DMN and SVAN tended to have an increase in connectivity with other networks, while FPCN showed mainly a decrease in within-network connectivity strength.

Contrary to a recently published longitudinal study with comparable age range (Ng et al., 2016), we did not show an increase in the DMN-FPCN connectivity. These authors showed a U-shaped trajectory in the functional integration between these networks, with a turning point around 65-70 years. However, they performed their calculations on data with global signal regression, including both positive and negative connections, contrary to us including only positive connections, which could have resulted in the present differences.

Nonetheless, we did show an increase in DMN-SVAN between-network connectivity strength. This decrease in the segregation between the default mode and the attention networks is in line with previously reported results in cross-sectional studies (Ferreira et al., 2016; Spreng et al., 2016).

The DMN is associated with internally directed cognition (i.e. mind-wandering, self-referential processing, episodic memory; Buckner, 2008, 2013), as opposed to FPCN/DAN and SVAN networks, which are involved in top-down goal directed and stimulus-driven bottom-up control of attention, respectively (Uddin, 2015; Menon & Uddin, 2010). Studies with healthy subjects suggest that these networks contain hub nodes which are crucial for the whole brain network connectivity and are important for optimal cognitive functioning (van den Heuvel et al., 2013). In particular, brain regions from the salience network have been hypothesized to have a crucial role in switching and coordinating between the DMN and FPCN across tasks and stimulus modalities, to facilitate the allocation of attentional and working memory resources when salient stimuli is detected (Uddin, 2015; Menon & Uddin, 2010).

In fact, the connectivity profile of the salience network, including the between-network connectivity with the DMN, has been labeled as the hallmark of aging in several studies (La Corte et al., 2016, Onoda et al., 2012; He et al., 2014).

Here, the salience ventral attention network showed a cross-sectional U-shaped trajectory, as younger elderly showed a 4-year decrease in the functional segregation, and older elderly (75 years and upwards) an increase over the same interval. Although speculative, this change could be interpreted as a possible compensatory mechanism in older participants as a response to this initial decrease seen in younger participants.

In contrast, the dorsal attention network did not exhibit statistically significant decline in segregation, and some research suggests that this network is less vulnerable to aging effects as opposed to other associative networks (Grady et al., 2016; Chan et al., 2014). However, our results did imply that older participants have higher DMN-DAN functional connectivity (not significant after multiple comparison correction), which does corroborate earlier findings of decreased segregation between the DMN and attention networks. Therefore, it is possible that changes in functional segregation of DAN happen much slower and cannot be detected over a 4-year time span.

Contrary to most previous cross-sectional studies (Damoiseaux, 2017; Ferreira et al., 2016), we did not find a decrease in the within-network connectivity strength in the default mode network. Moreover, ~~most models did not show a cross-sectional effect of age nor a significant interaction between age at baseline and time,~~ only two (i.e. DMN and SVAN) models showed cross-

sectional effects of age and one model found support for an interaction between age & time (i.e. SVAN), pointing to a more homogenous trajectory between participants of different ages in our sample (e.g. 65-year-old versus 80-year-old subjects). At first glance this might seem contradictory to the existing literature, however, this may be due to a narrower age range of subjects in our sample (i.e. 64-87 years), as opposed to previous studies which have assessed functional organization patterns across a wider age range incorporating both young and older participants. Also, our sample has more participants in the lower age range (i.e. 65-75 years old), which could have introduced bias into the statistical analysis.

When comparing our findings with the ones from past longitudinal studies, the decline in the segregation of the associative networks matches previous results (Chong et al., 2019).

However, specific within- and between-network connectivity patterns seem to be more heterogeneous across longitudinal research (Ng et al., 2016; Staffaroni et al., 2018; Salami et al., 2016, Persson et al., 2014). Specifically, some longitudinal studies on the functional connectivity of DMN suggest a decrease (Ng et al., 2016), while others show nonlinear change (Staffaroni et al., 2018) or point to an absence of connectivity change (Persson et al., 2014) across time.

Nonetheless, the advantage of longitudinal over cross-sectional studies is that they are able to model intra-individual change as opposed to cross-sectional comparison, in which we can only investigate inter-individual differences, with a risk of confounding aging effects with cohort effects (Schaie & Hofer, 2001). This is especially important in the context of functional segregation, as past research has shown that functional brain organization is influenced by non-neural factors such as the socio-economic status (Chan et al., 2018).

Accordingly, Chong et al., 2019 conducted both longitudinal (i.e. within-subject change) and cross-sectional analysis (group comparison between younger and older participants) and found different results between these two approaches. The longitudinal analysis showed more focal network changes in comparison to cross-sectional analyses which showed a nonspecific age-related decrease in segregation across all modules.

In contrary to a decline in segregative properties of other networks, and opposed to recent results of Chong et al., 2019, our findings showed an increase in functional segregation of the limbic network over the 4-year interval, which encompassed both an increase in within- and between-

network connectivity. In line with these results, some cross-sectional research demonstrated an absence of age-related decline in emotion/limbic networks (i.e. orbitofrontal cortex, temporal pole) in contrast to cognitive networks which are more vulnerable to aging effects (Nashiro et al., 2017). This has been related to preserved emotional function and an increase in emotional regulation in older age (Nashiro, Sakaki & Mather, 2012; Scheibe & Carstensen, 2010).

Finally, we wanted to test the significance of change in resting state functional segregation for offline (out-of-scanner) cognitive performance.

Reduced functional network segregation at rest has been associated with lower socio-economic status (Chan et al., 2018), poorer cognitive performance across age (Chan, 2014; Grady et al., 2016, Iordan, 2018), while higher segregation has been related to greater cognitive improvement after training (Baniqued et al., 2018; Gallen et al., 2016).

In the present study, a decline in the segregation of the frontoparietal control network was associated with a decline in processing speed. Although this result did not survive the multiple comparisons correction and should be interpreted with caution, it is in line with previous studies suggesting a beneficial role of the functional specialization of brain networks.

The FPCN is thought to be involved in working memory, decision making, and top-down allocation of attentional resources in the context of goal directed-behavior (Marek & Dosenbach, 2018; Cole et al., 2014a). This network has also been suggested to serve as a flexible hub which alters its functional connectivity with other networks based on the specific task (similar to the salience network), which allows it to coordinate between different networks during task performance (Cole et al., 2014a).

Previous research suggests high correspondence between task and rest-based functional connectivity in healthy subjects (Cole et al., 2014b), and implies that the aging-related dedifferentiation is indeed related to lower activation selectivity across corresponding network nodes during task conditions (Chan et al., 2017). Thus, we assume that reduced segregation at rest reflects the age-related changes in task activation and cognitive function, perhaps related to difficulties of the “dedifferentiated” FPCN to initiate and switch activity in relevant functional systems leading to lower ability to engage in goal-directed behavior and a decline in processing speed.

~~Further, we showed a potential benefitial impact of higher functional segregation of the limbic network, as there was less decline in verbal learning and memory at higher rates of increase in the segregation of this network. Although speculative, it is possible that the enhanced segregation of limbic network is related to a compensatory mechanism (Fjell et al., 2015) or an increase in emotional regulation and that this perserved emotional function is able to facilitate older participant's memory (Nashiro and Mather, 2011). Moreover, these findings are compatible with previous research showing higher cortical thickness in the coresponding limbic regions, such as the temporal pole and orbitofrontal cortex, in participants with better verbal learning and memory performance (Engvig et al., 2010; Wolk and Dickerson, 2011).~~

Finally, despite using a similar definition of cognitive domains as in the present study, a recent longitudinal study (Chong et al., 2019) did not show a significant change-change association between processing speed ~~verbal memory~~ and changes in functional segregation. Thus, future longitudinal studies are needed to further elucidate the brain-cognition relationship in the context of healthy aging.

Methodological considerations and limitations

It is worth noting that the results remained largely consistent after several validation analyses controlling for other covariates (i.e. motion, mean connectivity, ~~gray matter volume~~ cortical thickness) or differences in methodological choices (i.e. global signal regression, data filtering, parcellation scheme).

However, there are still some considerations that should be taken into account.

In our main analysis, we defined our networks according to a parcellation (Schaefer et al., 2018;) that has a network assignment (Yeo et al., 2011) commonly used in studies on healthy aging (Ng et al., 2016; Betzel et al., 2014). We then repeated our analysis using two other functional atlases that had similar, but not overlapping, resting-state network assignments. In these validation analyses, there was a significant decline in the functional segregation of the DMN, independently of network definition, while the SVAN (reproduced in one atlas) and FPCN were more contingent on the choice of the parcellation scheme. To better understand this discrepancy between the findings across parcellations, one would need to conduct analyses on a regional

level, which is out of the scope of this study. However, future studies are encouraged to investigate this in more detail.

Next, the application of global signal regression (GSR) has remained somewhat controversial as the primary concern regarding this approach is that it could possibly: a) remove neural information that might be present in the global signal; and (due to its mathematical characteristics) that it b) shifts the distribution of functional connectivity strength from predominantly positive to both positive and negative connectivity (Murphy & Fox, 2017). Regarding the first issue, it has been pointed out that the global signal consists primarily of signal of nonneural origin related to movement- and physiology-induced noise (Power et al., 2017). In fact, a potential confound in aging studies is motion, as older adults show greater amounts of head movement (Mowinckel et al., 2012) which has also been related to artefactually altered connectivity patterns (Power et al., 2012; Satterthwaite et al., 2013). In accordance, it has been shown that GSR, and more specifically the 36-parameter regression model that we have applied here (Ciric et al., 2017), is an efficient denoising strategy, especially in reducing the influence of movement (Satterthwaite et al., 2019). Nonetheless, due to ambiguity in the qualitative interpretation of negative correlations in the presence of global signal regression (Power et al. 2017; Fox and Murphy, 2016), and in accord with previous studies that used the segregation measure with GSR (Chan et al. 2014, 2017; Han et al., 2018; Chong et al., 2019), we decided to exclude these connections. Nonetheless, validation analyses with data without global signal regression, indeed revealed similar results to the main findings, further supporting the choice of GSR in the preprocessing of rs-fMRI.

A further issue relates to the observation that regions in the limbic network (i.e. OFC and temporal pole) tend to have lower BOLD signal-to-noise ratio (SNR) in comparison to other resting state networks and can thus suffer from signal loss (Yeo et al., 2011). In our analyses we compared summarized average measures on a network-level in order to reduced the influence of low SNR present in some but not all voxels within the limbic network.

Besides the above-mentioned issues, which primarily regard options in MRI data processing, it should be noted that, although the sample remained physically and mentally healthy across follow-up according to general health indicators, we cannot fully exclude that a small percentage

of the study population deviated from healthy cognitive aging trajectories given that our study design did not contain comprehensive clinical assessments besides the MMSE.

Furthermore, with respect to statistical modeling, we would like to point out that we fit hypothesis-driven "maximal" LMEMs, to investigate the change in functional segregation and cognition, that contained random slopes for time, regardless of the estimated variance of the random slopes (Barr et al., 2013). However, the (near-zero) variance in the random effects structure did not have an important influence on the significance tests of the fixed effects, thus not changing the interpretation of the results in comparison to random intercept models. Importantly, the extraction of random slopes for the effect of time allowed us to test associations between individual rates of change in brain and cognition.

Finally, future studies should investigate the association between the resting state functional segregation and performance in other cognitive domains, as some studies suggest that this relationship also depends on task complexity and cognitive load (Yue et al., 2017).

Conclusions

Using a network-based metric which summarizes the within- to between-network connectivity, we showed a significant change in the functional segregation of the default mode, frontoparietal control, salience ventral attention and limbic networks. In contrast, the dorsal attention, somatomotor and visual networks did not have any significant change in overall segregation over the 4-year time interval. The rate of change in the functional segregation of the frontoparietal control network ~~and the limbic network~~ was associated with the rate of change in processing speed, suggesting stronger cognitive decline at higher rates of decrease in the segregation of this network.~~ and verbal learning and memory, respectively~~. Finally, our findings highlight the importance of the segregation index as a neural marker of aging-related changes in network organization and cognitive function.

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Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Authorship

SM, FL, and LJ contributed to the design, set-up, maintenance and support of the Longitudinal Healthy Aging Brain (LHAB) database. BM performed the data analysis and wrote the first draft of the manuscript. All authors discussed the results, contributed to manuscript revision, read and approved the submitted version.

Data Availability Statement

The data for this manuscript are not publicly available. Since data collection was started in 2011, when public data sharing and open science were not yet widely discussed, the used consent does not allow for the public sharing of the data. We are currently working on a solution for this matter. At the moment data can only be accessed via collaborations with the URPP Dynamics of Healthy Aging.

References

Fischl, B., 2012. FreeSurfer. Neuroimage, 62(2), 774–781. https://doi.org/10.1016/j.neuroimage.2012.01.021

Power, J.D., Cohen, A.L., Nelson, S.M., Wig, G.S., Barnes, K.A., Church, J.A., Vogel, A.C., Laumann, T.O., Miezin, F.M., Schlaggar, B.L., Petersen, S.E., 2011. Functional Network Organization of the Human Brain. Neuron, 72(4), 665-78. https://doi.org/10.1016/j.neuron.2011.09.006

Shirer, W.R., Ryali, S., Rykhlevskaia, E., Menon, V., Greicius, M.D., 2012. Decoding subject-driven cognitive states with whole-brain connectivity patterns. Cereb. Cortex 22, 158–165. https://doi.org/10.1093/cercor/bhr099

Altmann, A., Ng, B., Landau, S.M., Jagust, W.J., Greicius, M.D., 2015. Regional brain hypometabolism is unrelated to regional amyloid plaque burden. Brain 138, 3734-46. https://doi.org/10.1093/brain/awv278

769 Barr, D.J., Levy, R., Scheepers, C., Tily, H.J., 2013. Random effects structure for confirmatory
770 hypothesis testing: Keep it maximal. J. Mem. Lang.
771 https://doi.org/10.1016/j.jml.2012.11.001

772 Engvig, A., Fjell, A.M., Westlye, L.T., Moberget, T., Sundseth, O., Larsen, V.A., Walhovd,
773 K.B., 2010. Effects of memory training on cortical thickness in the elderly. Neuroimage
774 52(4), 1667-76. https://doi.org/10.1016/j.neuroimage.2010.05.041

775 Wolk, D.A., Dickerson, B.C., 2011. Fractionating verbal episodic memory in Alzheimer's
776 disease. Neuroimage 54(2), 1530-9. https://doi.org/10.1016/j.neuroimage.2010.09.005

777 Mowinckel, A.M., Espeseth, T., Westlye, L.T., 2012. Network-specific effects of age and in-
778 scanner subject motion: A resting-state fMRI study of 238 healthy adults. Neuroimage
779 63(3), 1364-73. https://doi.org/10.1016/j.neuroimage.2012.08.004

780 Salami, A., Wahlin, A., Kaboodvand, N., Lundquist, A., Nyberg, L., 2016. Longitudinal
781 Evidence for Dissociation of Anterior and Posterior MTL Resting-State Connectivity in
782 Aging: Links to Perfusion and Memory. Cereb. Cortex. 26(10), 3953-3963.
783 https://doi.org/10.1093/cercor/bhw233

784 Persson, J., Pudas, S., Nilsson, L.G., Nyberg, L., 2014. Longitudinal assessment of default-mode
785 brain function in aging. Neurobiol. Aging 35(9), 2107-17.
786 https://doi.org/10.1016/j.neurobiolaging.2014.03.012

787 Chong, J.S.X., Ng, K.K., Tandi, J., Wang, C., Poh, J.H., Lo, J.C., Chee, M.W.L., Zhou J.H.
788 2019.
789 Longitudinal Changes in the Cerebral Cortex Functional Organization of Healthy Elderly. The
790 Journal of Neuroscience. 39(28), 5534 –5550.
791
792 Antonenko, D., Flöel, A., 2013. Healthy aging by staying selectively connected: A mini-review.
793 Gerontology 60, 3–9. https://doi.org/10.1159/000354376

794 Aster, M., Neubauer, A., Horn, R., 2006. Wechsler Intelligenztest für Erwachsene WIE.
795 Deutschsprachige Bearbeitung und Adaption des WAIS-III von David Wechsler.
796 Frankfurt/Main, Ger. Harcourt Test Serv.
797 https://doi.org/http://dx.doi.org/10.1038/srep18573

798 Avants, B.B., Epstein, C.L., Grossman, M., Gee, J.C., 2008. Symmetric diffeomorphic image
799 registration with cross-correlation: Evaluating automated labeling of elderly and
800 neurodegenerative brain. Med. Image Anal. 12, 26–41.
801 https://doi.org/10.1016/j.media.2007.06.004

802 Baniqued, P.L., Gallen, C.L., Voss, M.W., Burzynska, A.Z., Wong, C.N., Cooke, G.E., Duffy,
803 K., Fanning, J., Ehlers, D.K., Salerno, E.A., Aguiñaga, S., McAuley, E., Kramer, A.F.,
804 D'Esposito, M., 2018. Brain network modularity predicts exercise-related executive

function gains in older adults. Front. Aging Neurosci. 9, 1–17. https://doi.org/10.3389/fnagi.2017.00426

Bates, D., Mächler, M., Bolker, B., Walker, S., 2014. Fitting Linear Mixed-Effects Models using lme4 67. https://doi.org/10.18637/jss.v067.i01

Betzel, R.F., Byrge, L., He, Y., Goñi, J., Zuo, X.N., Sporns, O., 2014. Changes in structural and functional connectivity among resting-state networks across the human lifespan. Neuroimage 102, 345–357. https://doi.org/10.1016/j.neuroimage.2014.07.067

Buckner, R.L., 2013. The brain's default network: Origins and implications for the study of psychosis. Dialogues Clin. Neurosci. 15, 351–358.

Buckner, R.L., Andrews-Hanna, J.R., Schacter, D.L., 2008. The Brain's Default Network. Ann. N. Y. Acad. Sci. 1124, 1–38. https://doi.org/10.1196/annals.1440.011

Bullmore, E., Sporns, O., 2012. The economy of brain network organization. Nat. Rev. Neurosci. 13, 336–349. https://doi.org/10.1038/nrn3214

Chan, M.Y., Alhazmi, F.H., Park, D.C., Savalia, N.K., Wig, G.S., 2017. Resting-State Network Topology Differentiates Task Signals across the Adult Life Span. J. Neurosci. 37, 2734–2745. https://doi.org/10.1523/JNEUROSCI.2406-16.2017

Chan, M.Y., Na, J., Agres, P.F., Savalia, N.K., Park, D.C., Wig, G.S., 2018. Socioeconomic status moderates age-related differences in the brain's functional network organization and anatomy across the adult lifespan. Proc. Natl. Acad. Sci. https://doi.org/10.1073/pnas.1714021115

Chan, M.Y., Park, D.C., Savalia, N.K., Petersen, S.E., Wig, G.S., 2014. Decreased segregation of brain systems across the healthy adult lifespan. Proc. Natl. Acad. Sci. 111, E4997–E5006. https://doi.org/10.1073/pnas.1415122111

Ciric, R., Wolf, D.H., Power, J.D., Roalf, D.R., Baum, G.L., Ruparel, K., Shinohara, R.T., Elliott, M.A., Eickhoff, S.B., Davatzikos, C., Gur, R.C., Gur, R.E., Bassett, D.S., Satterthwaite, T.D., 2017. Benchmarking of participant-level confound regression strategies for the control of motion artifact in studies of functional connectivity. Neuroimage. https://doi.org/10.1016/j.neuroimage.2017.03.020

Cohen, J., 1988. Statistical power analysis for the behavioral sciences, (2nd ed.). Hillsdale, NJ: Lawrence Erlbaum Associates, Publishers. https://doi.org/10.1234/12345678

Cole, M.W., Bassett, D.S., Power, J.D., Braver, T.S., Petersen, S.E., 2014. Intrinsic and task-evoked network architectures of the human brain. Neuron. https://doi.org/10.1016/j.neuron.2014.05.014

838 Cole, M.W., Repovš, G., Anticevic, A., 2014. The Frontoparietal Control System. Neurosci. 20,
839 652–664. https://doi.org/10.1177/1073858414525995

840 Cox, R.W., 1996. AFNI: Software for analysis and visualization of functional magnetic
841 resonance neuroimages. Comput. Biomed. Res. 29, 162–173.
842 https://doi.org/10.1006/cbmr.1996.0014

843 Damoiseaux, J.S., 2017. Effects of aging on functional and structural brain connectivity.
844 Neuroimage 160, 32–40. https://doi.org/10.1016/j.neuroimage.2017.01.077

845 Ekstrom, R.B.R., French, J.J.W.J.J.W., Harman, H.H., Dermen, D., 1976. Manual for kit of
846 factor-referenced cognitive tests. Princet. NJ Educ. Test. Serv.
847 https://doi.org/10.1073/pnas.0506897102

848 Esteban, O., Markiewicz, C.J., Blair, R.W., Moodie, C.A., Isik, A.I., Erramuzpe, A., Kent, J.D.,
849 Goncalves, M., DuPre, E., Snyder, M., Oya, H., Ghosh, S.S., Wright, J., Durnez, J.,
850 Poldrack, R.A., Gorgolewski, K.J., 2019. fMRIPrep: a robust preprocessing pipeline for
851 functional MRI. Nat. Methods. https://doi.org/10.1038/s41592-018-0235-4

852 Ferreira, L.K., Regina, A.C.B., Kovacevic, N., Martin, M.D.G.M., Santos, P.P., Carneiro,
853 C.D.G., Kerr, D.S., Amaro, E., Mcintosh, A.R., Busatto, G.F., 2016. Aging effects on
854 whole-brain functional connectivity in adults free of cognitive and psychiatric disorders.
855 Cereb. Cortex 26, 3851–3865. https://doi.org/10.1093/cercor/bhv190

856 Fjell, A.M., Sneve, M.H., Grydeland, H., Storsve, A.B., de Lange, A.M.G., Amlien, I.K.,
857 Røgeberg, O.J., Walhovd, K.B., 2015. Functional connectivity change across multiple
858 cortical networks relates to episodic memory changes in aging. Neurobiol. Aging 36, 3255–
859 3268. https://doi.org/10.1016/j.neurobiolaging.2015.08.020

860 Folstein, M.F., Folstein, S.E., McHugh, P.R., 1975. “Mini-mental state”. A practical method for
861 grading the cognitive state of patients for the clinician. J. Psychiatr. Res. 12(3), 189–198.
862 https://doi.org/10.1016/0022-3956(75)90026-6

863 Fonov, V., Almli, C., Evans, A., Collins, D., McKinstry, R., 2009. Unbiased nonlinear average
864 age-appropriate brain templates from birth to adulthood. Neuroimage 47, S102.
865 https://doi.org/10.1016/s1053-8119(09)70884-5

866 Fornito, A., Zalesky, A., Breakspear, M., 2015. The connectomics of brain disorders. Nat. Rev.
867 Neurosci. 16, 159–172. https://doi.org/10.1038/nrn3901

868 Fornito, A., Zalesky, A., Bullmore, E.T., 2016. Fundamentals of Brain Network Analysis,
869 Fundamentals of Brain Network Analysis. https://doi.org/10.1016/C2012-0-06036-X

870 Gallen, C.L., Baniqued, P.L., Chapman, S.B., Aslan, S., Keebler, M., Didehbani, N., D’Esposito,
871 M., 2016. Modular brain network organization predicts response to cognitive training in
872 older adults. PLoS One. https://doi.org/10.1371/journal.pone.0169015

Geerligs, L., Renken, R.J., Saliasi, E., Maurits, N.M., Lorist, M.M., 2015. A Brain-Wide Study of Age-Related Changes in Functional Connectivity. Cereb. Cortex 25, 1987–1999. https://doi.org/10.1093/cercor/bhu012

Geerligs, L., Tsvetanov, K.A., Cam-CAN, Henson, R.N., 2017. Challenges in measuring individual differences in functional connectivity using fMRI: The case of healthy aging. Hum. Brain Mapp. 38, 4125–4156. https://doi.org/10.1002/hbm.23653

Gorgolewski, K., Madison, C., Burns, C.D., Clark, D., Halchenko, Y.O., Waskom, M.L., Ghosh, S.S., 2011. Nipype: A Flexible, Lightweight and Extensible Neuroimaging Data Processing Framework in Python. Front. Neuroinform. 5. https://doi.org/10.3389/fninf.2011.00013

Grady, C., Sarraf, S., Saverino, C., Campbell, K., 2016. Age differences in the functional interactions among the default, frontoparietal control, and dorsal attention networks. Neurobiol. Aging 41, 159–172. https://doi.org/10.1016/j.neurobiolaging.2016.02.020

Greve, D.N., Fischl, B., 2009. Accurate and robust brain image alignment using boundary-based registration. Neuroimage 48, 63–72. https://doi.org/10.1016/j.neuroimage.2009.06.060

Hallquist, M.N., Hwang, K., Luna, B., 2013. The nuisance of nuisance regression: Spectral misspecification in a common approach to resting-state fMRI preprocessing reintroduces noise and obscures functional connectivity. Neuroimage. https://doi.org/10.1016/j.neuroimage.2013.05.116

He, X., Qin, W., Liu, Y., Zhang, X., Duan, Y., Song, J., Li, K., Jiang, T., Yu, C., 2014. Abnormal salience network in normal aging and in amnestic mild cognitive impairment and Alzheimer's disease. Hum. Brain Mapp. 35, 3446–3464. https://doi.org/10.1002/hbm.22414

Heine, L., Soddu, A., G??mez, F., Vanhaudenhuyse, A., Tshibanda, L., Thonnard, M., Charland-Verville, V., Kirsch, M., Laureys, S., Demertzi, A., Gómez, F., Vanhaudenhuyse, A., Tshibanda, L., Thonnard, M., Charland-Verville, V., Kirsch, M., Laureys, S., Demertzi, A., 2012. Resting state networks and consciousness Alterations of multiple resting state network connectivity in physiological, pharmacological, and pathological consciousness states. Front. Psychol. 3, 1–12. https://doi.org/10.3389/fpsyg.2012.00295

Helmstaedter, C., & Durwen, H. F., 1990. The Verbal Learning and Retention Test. A useful and differentiated tool in evaluating verbal memory performance. *Schweizer Archiv Fur Neurologie Und Psychiatrie. Archives Suisses de Neurologie et de Psychiatrie. Archivio Svizzero Di Neurologia e Psichiatria*, 141(1), 21–30.

Hoffman, L., Hofer, S.M., Sliwinski, M.J., 2011. On the confounds among retest gains and age-cohort differences in the estimation of within-person change in longitudinal studies: A simulation study. Psychol. Aging. https://doi.org/10.1037/a0023910

Horn, W. (1983). *Leistungsprüfsystem L-P-S.* (2nd ed.). Göttingen: Hogrefe.

908 Iordan, A.D., Cooke, K.A., Moored, K.D., Katz, B., Buschkuehl, M., Jaeggi, S.M., Jonides, J.,
909 Peltier, S.J., Polk, T.A., Reuter-Lorenz, P.A., 2018. Aging and network properties: Stability
910 over time and links with learning during working memory training. Front. Aging Neurosci.
911 https://doi.org/10.3389/fnagi.2017.00419

912 James, G.A., Kearney-Ramos, T.E., Young, J.A., Kilts, C.D., Gess, J.L., Fausett, J.S., 2016.
913 Functional independence in resting-state connectivity facilitates higher-order cognition.
914 Brain Cogn. 105, 78–87. https://doi.org/10.1016/j.bandc.2016.03.008

915 Jenkinson, M., Bannister, P., Brady, M., Smith, S., 2002. Improved optimization for the robust
916 and accurate linear registration and motion correction of brain images. Neuroimage.

917 Kuznetsova, A., Brockhoff, P.B., Christensen, R.H.B., 2017. lmerTest Package: Tests in Linear
918 Mixed Effects Models. J. Stat. Softw. https://doi.org/10.18637/jss.v082.i13

919 La Corte, V., Sperduti, M., Malherbe, C., Vialatte, F., Lion, S., Gallarda, T., Oppenheim, C.,
920 Piolino, P., 2016. Cognitive decline and reorganization of functional connectivity in healthy
921 aging: The pivotal role of the salience network in the prediction of age and cognitive
922 performances. Front. Aging Neurosci. 8, 1–12. https://doi.org/10.3389/fnagi.2016.00204

923 Leys, C., Ley, C., Klein, O., Bernard, P., Licata, L., 2013. Detecting outliers: Do not use
924 standard deviation around the mean, use absolute deviation around the median. J. Exp. Soc.
925 Psychol. 49(4), 764–766. https://doi.org/10.1016/j.jesp.2013.03.013

926 Li, H.J., Hou, X.H., Liu, H.H., Yue, C.L., Lu, G.M., Zuo, X.N., 2015. Putting age-related task
927 activation into large-scale brain networks: A meta-analysis of 114 fMRI studies on healthy
928 aging. Neurosci. Biobehav. Rev. 57, 156–174.
929 https://doi.org/10.1016/j.neubiorev.2015.08.013

930 Li, K.Z.H., Lindenberger, U., 2002. Relations between aging sensory/sensorimotor and cognitive
931 functions. Neurosci. Biobehav. Rev. 26, 777–783. https://doi.org/10.1016/S0149-
932 7634(02)00073-8

933 Lindenberger, U., Singer, T., Baltes, P.B., 2002. Longitudinal selectivity in aging populations:
934 Separating mortality-associated versus experimental components in the Berlin Aging Study
935 (BASE). Journals Gerontol. - Ser. B Psychol. Sci. Soc. 57B(6), 474–482
936 https://doi.org/10.1093/geronb/57.6.P474

937 Marek, S., Dosenbach, N.U.F., 2018. The frontoparietal network: function, electrophysiology,
938 and importance of individual precision mapping. Dialogues Clin. Neurosci. 20, 133–140.

939 Meier, T.B., Desphande, A.S., Vergun, S., Nair, V.A., Song, J., Biswal, B.B., Meyerand, M.E.,
940 Birn, R.M., Prabhakaran, V., 2012. Support vector machine classification and
941 characterization of age-related reorganization of functional brain networks. Neuroimage 60,
942 601–613. https://doi.org/10.1016/j.neuroimage.2011.12.052

943 Menon, V., Uddin, L.Q., 2010. Saliency, switching, attention and control: a network model of
944 insula function. Brain Struct. Funct. https://doi.org/10.1007/s00429-010-0262-0

945 Mérillat, S., Zöllig, J., Martin, M., Eschen, A., Röcke, C., Jäncke, L., 2011. Plasticity and
946 Imaging Research in Healthy Aging: Core Ideas and Profile of the International Normal
947 Aging and Plasticity Imaging Center (INAPIC). Gerontology 57, 190–192.
948 https://doi.org/10.1159/000324307

949 Muller, A.M., Mérillat, S., Jäncke, L., 2016. Small changes, but huge impact? The right anterior
950 insula's loss of connection strength during the transition of old to very old age. Front. Aging
951 Neurosci. 8, 1–20. https://doi.org/10.3389/fnagi.2016.00086

952 Murphy, K., Fox, M.D., 2017. Towards a consensus regarding global signal regression for
953 resting state functional connectivity MRI. Neuroimage.
954 https://doi.org/10.1016/j.neuroimage.2016.11.052

955 Nashiro, K., Mather, M., 2011. Effects of emotional arousal on memory binding in normal aging
956 and Alzheimer's disease. Am. J. Psychol. 124, 301–312.
957 https://doi.org/10.5406/amerjpsyc.124.3.0301

958 Nashiro, K., Sakaki, M., Braskie, M.N., Mather, M., 2017. Resting-state networks associated
959 with cognitive processing show more age-related decline than those associated with
960 emotional processing. Neurobiol. Aging.
961 https://doi.org/10.1016/j.neurobiolaging.2017.03.003

962 Nashiro, K., Sakaki, M., Mather, M., 2012. Age differences in brain activity during emotion
963 processing: Reflections of age-related decline or increased emotion regulation? Gerontology
964 58, 156–163. https://doi.org/10.1159/000328465

965 Ng, K.K., Lo, J.C., Lim, J.K.W., Chee, M.W.L., Zhou, J., 2016. Reduced functional segregation
966 between the default mode network and the executive control network in healthy older
967 adults: A longitudinal study. Neuroimage 133, 321–330.
968 https://doi.org/10.1016/j.neuroimage.2016.03.029

969 Ng, K.K., Qiu, Y., Lo, J.C.Y., Koay, E.S.C., Koh, W.P., Chee, M.W.L., Zhou, J., 2018.
970 Functional segregation loss over time is moderated by APOE genotype in healthy elderly.
971 Hum. Brain Mapp. https://doi.org/10.1002/hbm.24036

972 Onoda, K., Ishihara, M., Yamaguchi, S., 2012. Decreased functional connectivity by aging is
973 associated with cognitive decline. J. Cogn. Neurosci. https://doi.org/10.1162/jocn_a_00269

974 Oschwald, J., Mérillat, S., Liem, F., Röcke, C., Martin, M., & Jäncke, L. 2019. Lagged coupled
975 changes between white matter microstructure and processing speed in healthy aging: a
976 longitudinal investigation. Manuscript submitted for publication.

977 Reitan, R.M., Wolfson, D., 2004. The Trail Making Test as an initial screening procedure for
978 neuropsychological impairment in older children, in: Archives of Clinical
979 Neuropsychology,-19(2), 281–288. https://doi.org/10.1016/S0887-6177(03)00042-8

980 Roski, C., Caspers, S., Langner, R., Laird, A.R., Fox, P.T., Zilles, K., Amunts, K., Eickhoff,
981 S.B., 2013. Adult age-dependent differences in resting-state connectivity within and
982 between visual-attention and sensorimotor networks. Front. Aging Neurosci. 5, 1–10.
983 https://doi.org/10.3389/fnagi.2013.00067

984 Schaefer, A., Kong, R., Gordon, E.M., Laumann, T.O., Zuo, X.-N., Holmes, A.J., Eickhoff, S.B.,
985 Yeo, B.T.T., 2018. Local-Global Parcellation of the Human Cerebral Cortex from Intrinsic
986 Functional Connectivity MRI. Cereb. Cortex. https://doi.org/10.1093/cercor/bhx179

987 Schaie, K.W., Hofer, S.M., 2001. Longitudinal studies in aging research. In: Birren, JE.; Schaie,
988 KW., editors. Handbook of the Psychology of Aging. fifth. Academic Press; San Diego:53-
989 77.

990 Scheibe, S., Carstensen, L.L., 2010. Emotional aging: Recent findings and future trends. Journals
991 Gerontol. - Ser. B Psychol. Sci. Soc. Sci. 65 B, 135–144.
992 https://doi.org/10.1093/geronb/gbp132

993 Seidler, R., Erdeniz, B., Koppelmans, V., Hirsiger, S., Mérillat, S., Jäncke, L., 2015.
994 Associations between age, motor function, and resting state sensorimotor network
995 connectivity in healthy older adults. Neuroimage 108, 47–59.
996 https://doi.org/10.1016/j.neuroimage.2014.12.023

997 Seidler, R.D., Bernard, J.A., Burutolu, T.B., Fling, B.W., Gordon, M.T., Gwin, J.T., Kwak, Y.,
998 Lipps, D.B., 2010. Motor control and aging: Links to age-related brain structural,
999 functional, and biochemical effects. Neurosci. Biobehav. Rev. 34, 721–733.
1000 https://doi.org/10.1016/j.neubiorev.2009.10.005

1001 Smitha, K.A., Akhil Raja, K., Arun, K.M., Rajesh, P.G., Thomas, B., Kapilamoorthy, T.R.,
1002 Kesavadas, C., 2017. Resting state fMRI: A review on methods in resting state connectivity
1003 analysis and resting state networks. Neuroradiol. J.
1004 https://doi.org/10.1177/1971400917697342

1005 Song, J., Meyerand, M.E., Meier, T.B., Birn, R.M., Prabhakaran, V., Nair, V.A., Boly, M., 2014.
1006 Age-Related Reorganizational Changes in Modularity and Functional Connectivity of
1007 Human Brain Networks. Brain Connect. 4, 662–676.
1008 https://doi.org/10.1089/brain.2014.0286

1009 Spreng, R.N., Stevens, W.D., Viviano, J.D., Schacter, D.L., 2016. Attenuated anticorrelation
1010 between the default and dorsal attention networks with aging: evidence from task and rest.
1011 Neurobiol. Aging 45, 149–160. https://doi.org/10.1016/j.neurobiolaging.2016.05.020

1012 Staffaroni, A.M., Brown, J.A., Casaletto, K.B., Elahi, F.M., Deng, J., Neuhaus, J., Cobigo, Y.,
1013 Mumford, P.S., Walters, S., Saloner, R., Karydas, A., Coppola, G., Rosen, H.J., Miller,
1014 B.L., Seeley, W.W., Kramer, J.H., 2018. The Longitudinal Trajectory of Default Mode
1015 Network Connectivity in Healthy Older Adults Varies As a Function of Age and Is
1016 Associated with Changes in Episodic Memory and Processing Speed. J. Neurosci.
1017 https://doi.org/10.1523/JNEUROSCI.3067-17.2018

1018 Stam, C.J., 2014. Modern network science of neurological disorders. Nat. Rev. Neurosci. 15,
1019 683–695. https://doi.org/10.1038/nrn3801

1020 Tustison, N.J., Avants, B.B., Cook, P.A., Zheng, Y., Egan, A., Yushkevich, P.A., Gee, J.C.,
1021 2010. N4ITK: Improved N3 bias correction. IEEE Trans. Med. Imaging.
1022 https://doi.org/10.1109/TMI.2010.2046908

1023 Uddin, L.Q., 2015. Salience processing and insular cortical function and dysfunction. Nat. Rev.
1024 Neurosci. 16, 55–61. https://doi.org/10.1038/nrn3857

1025 Van Den Heuvel, M.P., Pol, H.E.H., 2011. Exploring the brain network: A review on resting-
1026 state fMRI functional connectivity. Psiquiatr. Biol. 18, 28–41.
1027 https://doi.org/10.1016/j.psiq.2011.05.001

1028 Von Aster, M., Neubauer, A., & Horn, R., 2006. *Wechsler-Intelligenztest für Erwachsene (WIE).*
1029 *Deutschsprachige Bearbeitung und Adaptation des WAIS-III von David Wechsler*.
1030 Frankfurt: Harcourt Test Services.
1031
1032 Ware, J.E., Kosinski, M., Keller, S.D., 1996. A 12-Item Short-Form Health Survey: Construction
1033 of Scales and Preliminary Tests of Reliability and Validity. Med. Care.
1034 https://doi.org/10.1097/00005650-199603000-00003

1035 Whitfield-Gabrieli, S., Nieto-Castanon, A., 2012. Conn: A Functional Connectivity Toolbox for
1036 Correlated and Anticorrelated Brain Networks. Brain Connect. 2, 125–141.
1037 https://doi.org/10.1089/brain.2012.0073

1038 Wig, G.S., 2017. Segregated Systems of Human Brain Networks. Trends Cogn. Sci. 21, 981–
1039 996. https://doi.org/10.1016/j.tics.2017.09.006

1040 Yeo, B.T.T., Krienen, F.M., Sepulcre, J., Sabuncu, M.R., Lashkari, D., Hollinshead, M.,
1041 Roffman, J.L., Smoller, J.W., Zöllei, L., Polimeni, J.R., Fischl, B., Liu, H., Buckner, R.L.,
1042 2011. The organization of the human cerebral cortex estimated by intrinsic functional
1043 connectivity. J. Neurophysiol. https://doi.org/10.1152/jn.00338.2011

1044 Yue, Q., Martin, R.C., Fischer-Baum, S., Ramos-Nuñez, A.I., Ye, F., Deem, M.W., 2017. Brain
1045 modularity mediates the relation between task complexity and performance. J. Cogn.
1046 Neurosci. https://doi.org/10.1162/jocn_a_01142

1047 Zhang, Y., Brady, M., Smith, S., 2001. Segmentation of brain MR images through a hidden
1048 Markov random field model and the expectation-maximization algorithm. IEEE Trans.
1049 Med. Imaging 20, 45–57. https://doi.org/10.1109/42.906424

1050 Zöllig, J., Mérillat, S., Eschen, A., Röcke, C., Martin, M., Jäncke, L., 2011. Plasticity and
1051 Imaging Research in Healthy Aging: Core Ideas and Profile of the International Normal
1052 Aging and Plasticity Imaging Center (INAPIC). Gerontology, 57(2), 190–192.
1053 https://doi.org/10.1159/000324307